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Betaine improves growth, but does not induce whole body or hepatic palmitate oxidation in swine (*Sus scrofa domestica*)[☆]

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Abstract

Dietary betaine may reduce carcass fat in growing pigs. We explored the effects of betaine on short-term growth and in vivo and in vitro fatty acid oxidation. Pigs were housed in metabolism crates and fed diets containing either 0% (control), 0.125% or 0.5% betaine at 80% of ad libitum energy intake. Fatty acid oxidation was measured during intravenous infusions of 1-¹³C-palmitate and in hepatocytes incubated in the presence or absence of betaine and carnitine. CO₂ and palmitate isotopic enrichments were determined by mass spectrometry. Pigs consuming 0.125% and 0.5% betaine for at least 9 days had growth rates that were 38% and 12% greater than controls, respectively. Feed efficiency was also improved with betaine. Fasting increased palmitate oxidation rates 7–8-fold ($P < 0.01$), but betaine had no effect in either the fed or fasted state ($P > 0.1$). For hepatocytes, carnitine but not betaine enhanced palmitate oxidation. This response suggests that previously observed reduction in adipose accretion must be via a mechanism other than oxidation. Betaine had no effect on plasma non-esterified fatty acids or urea nitrogen. Under the confinement conditions in this study, dietary betaine improved animal growth responses, but it had no apparent effect on either whole body or hepatic fatty acid oxidation.

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1. Introduction

Betaine is a naturally occurring tertiary amine (trimethyl glycine), which is formed by the oxidation of choline, and it is present in most living

organisms. It is highly concentrated in the sugar beet, from which it is extracted as a byproduct during sugar production, and is readily available for use in animal feed (Virtanen, 1995). The use of betaine as an ingredient in livestock diets is not a new concept. It was initially introduced to the feed industry as a replacement for methionine and choline in poultry and fish diets, where it is presumed to act both as methyl donor and as an osmoprotectant (Kidd et al., 1997). In swine, it has been suggested that betaine reduces carcass fat deposition (Cadogan et al., 1993; Fernández-Fígares et al., 2002).

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While not the only source of labile methyl groups, betaine plays an integral role in 1-carbon metabolism. A methyl group from betaine is transferred to *S*-adenosylmethionine (SAM) via methionine. *S*-Adenosylmethionine, or 'active'-methionine, serves as the primary methyl donor for the biosynthesis of many compounds including carnitine, creatine, nucleic acids, neurotransmitters, phospholipids and hormones. Thus, betaine may be integrally involved in lipid metabolism via its role on phosphatidylcholine synthesis and in fatty acid oxidation because carnitine is required for transport of long chain fatty acids into mitochondria. In addition, Barak et al. have shown that betaine increases hepatic SAM and that betaine can both prevent and reverse alcohol-induced hepatotoxicity in laboratory rodents (Barak et al., 1994, 1997). A considerable portion of cellular methionine is activated by ATP to form SAM. This process may also be important in the coordinated regulation of partitioning of homocysteine (a potentially toxic intermediary metabolite), between de novo methionine synthesis (using a methyl group from betaine or folate), and catabolism through cystathionine (Selhub and Miller, 1992).

Addition of betaine in swine diets has increased during the last decade, but the experimental results for growth and carcass performance have not been consistent. Initial studies indicated that dietary betaine markedly decreased backfat thickness without influencing other growth parameters (Cadogan et al., 1993). More recent studies in finishing pigs, using various nutritional regimens and experimental conditions have also demonstrated decreases in some indicators of body fat (Lawrence et al., 2002) with a potential reduction in feed intake (Matthews et al., 2001a) under some conditions. In contrast, other studies in finishing pigs have reported minimal or no effects of betaine on growth, feed intake or body fat (Matthews et al., 1998; Overland et al., 1999). In young, feed-restricted pigs, total body composition analysis indicated that betaine was associated primarily with decreased carcass fat, and an increase in protein deposition, particularly at higher levels of betaine intake (Fernández-Fígares et al., 2002).

Much of the evidence from growth trials, including from our lab (Fernández-Fígares et al., 2002), suggests that betaine may indeed depress overall fat deposition, and this study focused on betaine's potential stimulatory effects on lipid oxidation as

a possible mechanism of action for fat reduction in growing pigs. Information on the role of betaine in altering lipid metabolism in the pig is limited to growth studies. For this study, our specific aims were to determine the effect of dietary betaine on long-chain fatty acid (palmitate) oxidation both in the whole body and in primary monolayer cultures of liver cells obtained from growing pigs.

2. Materials and methods

2.1. Diets and animals

Crossbred (Yorkshire×Landrace) female pigs (gilts) were used for all studies [(*Sus scrofa domestica*); from the Beltsville Agricultural Research Center (BARC) swine herd]. All animal care and use procedures were approved by the Beltsville Animal Care and Use Committee. From 20–30 kg of body mass, pigs were restrictively fed a corn–soybean meal based diet (1.2% lysine, 13.5 MJ/kg) at 80% estimated ad libitum consumption according to the Agricultural Research Council (ARC, 1981) formula (daily digestible energy intake, MJ = $0.80 \times 55 [1 - e^{-0.0204 \times BW}]$). Pigs to be used for in vivo fatty acid oxidation determinations were trained to breathe into a breath collection mask at this time. At 40 kg of body mass pigs were moved to metabolism crates and placed on the control diet (Table 1) for at least 1 week before being assigned to one of three treatment diets: control (0% betaine added to diet, wt./wt.), 0.125% betaine or 0.5% betaine. Pigs were fed treatment diets for at least 4 days before initiating the whole body fatty acid oxidation protocol in order to allow betaine–homocysteine methyltransferase levels to re-equilibrate (Finkelstein et al., 1983). Animals were weighed the day before the initiation of palmitate infusion studies in order to calculate the infusate dosage.

2.2. Growth parameters

Animals were weighed at least weekly and the amount of feed offered to each pig was adjusted once a week according to individual body mass based on the ARC equation shown above. All feed refusals were recorded. Average daily gain (growth rate) and feed intake were determined for all animals during the control diet period (8.4 ± 0.4 days) and then after initiation of the treatment diets. Animals were fed the treatment diets for

Table 1
Composition of basal diet^a

Ingredients	Percentage of diet
Corn	65.03
Soybean meal (48% CP)	18.29
Dried skim milk	12.00
Animal fat (lard)	1.00
Mineral–vitamin mix ^b	2.50
Dicalcium phosphate	1.08
L-Lysine	0.10
<i>Calculated nutrient composition, %</i>	
Crude protein (CP)	18.6
Fat	3.67
Metabolizable energy, MJ/kg diet	13.5
Calcium	0.97
Phosphorous	0.77
Lysine	1.20
Methionine	0.33
Methionine + Cysteine	0.63
Tryptophan	0.22
Threonine	0.75
Arginine	1.12
Isoleucine	0.76
Leucine	1.65
Histidine	0.45
Valine	0.91
Phenylalanine	0.90
Glycine	0.65
Choline	0.11

^a Diets prepared by United Feeds Inc., Sheridan, IN.

^b Provided the following amounts per kilogram of feed: Zn, 100.4 mg; Fe, 128.3 mg; Mn, 43.2 mg; Cu, 6.7 mg; I, 0.88 mg; Co, 0.34 mg; Se, 0.26 mg; vitamin A, 4000 IU; vitamin D, 800 IU; vitamin E, 17 IU; vitamin B12, 0.02 mg; vitamin K activity, 1.9 mg; riboflavin, 8.3 mg; D-Pantotenic acid, 21.3 mg; niacin, 43.0 mg; choline, 1088 mg; thiamine, 3.4 mg; pyridoxine, 6.9 mg; folic acid, 0.39 mg; biotin, 0.15 mg; Na, 2.6 g.

11.6 ± 0.5 days for the collection of growth and feed intake data. Growth and feed intake data were acquired before the initiation of infusion studies. The ratio of average daily feed intake and average daily gain (feed to gain ratio; F:G) was used as a measure of feed efficiency.

2.3. Catheterization and blood sampling

For all pigs, catheters were non-surgically placed in the jugular veins (via both medial auricular veins and the saphenous artery) under general anesthesia [2 mg xylazine and 2 mg telazol (Fort Dodge Laboratories, Inc., Fort Dodge, IA) per kg] to allow for simultaneous sampling and infusion in unanesthetized pigs (Wray-Cahen et al., 1993). Blood samples were obtained from the catheters

during the control diet period (at least 4 days after catheterization) and 1 week after initiation of treatment diet. Samples were collected in heparinized syringes and plasma was obtained after centrifugation (1800 × g, 15 min 4 °C) and frozen at −20 °C. Plasma non-esterified fatty acid (NEFA-C kit, Wako Diagnostics, Richmond, VA) and urea nitrogen (Sigma Diagnostic Kit No. 640; Sigma, St. Louis, MO) concentrations were determined for these samples and on frozen aliquots of plasma collected during the basal period before each isotope infusion. Kinetic measurements were made under steady-state fed or fasted conditions.

Fatty acid oxidation was determined using a method described by Wray-Cahen et al. (2001) using ¹³C-palmitate-methyl-β-cyclodextrin complex as a fatty acid tracer. The pigs receiving the control diet and the high level of betaine (0.5%) were studied under both fed and fasted conditions, on two separate, non-consecutive days. Animals on the infusion study consumed the treatment diets for 14 ± 3 days. For the in vivo fatty acid oxidation study, four pigs per treatment were infused during the fed state and five pigs per treatment were infused during the fasted state. Kinetic measurements were made under steady-state fed or fasted conditions. Under fed conditions, pigs received one-tenth of their daily ration, hourly, beginning 2 h before basal samples were collected and continuing throughout the collection period, so that the entire ration was consumed over a 9-h period. Under fasted conditions, collections began following an 18-h fast. Feed refusals during the sampling period were recorded.

2.4. Infusate preparation

[1-¹³C]-Palmitate (potassium salt; 99AP) was obtained from Mass Trace, Inc. (Woburn, MA). In the present investigation, [1-¹³C]-palmitate was bound to methyl-β-cyclodextrin (MCBD; Aldrich Chemical Co., Inc., Milwaukee, WI, USA; 33,261-5) at 3 g palmitate/100 g methyl-β-cyclodextrin in a 20% methyl-β-cyclodextrin solution, prepared in phosphate buffered saline (Wray-Cahen et al., 2001). The mixture was prepared at 50–60 °C and filtered through a 0.2 μm sterile filter into a sterile bottle and stored at 4 °C until infused. The final infusate enrichments (99.22 ± 0.06%) and palmitate concentrations were determined by gas chromatography-mass spectrometry (Metabolic Solutions, Inc.; Merrimack, NH, USA). The final

palmitate concentration after filtering the methyl- β -cyclodextrin infusate was $75.2 \pm 1.8\%$ of the calculated values. Values used in the palmitate oxidation calculations were those determined by mass spectrometry analysis.

2.5. Whole body fatty acid oxidation: infusion protocol

A 90 min primed-continuous (0.6 or 1.2 $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; fed or fasted conditions, respectively) infusion of $\text{NaH}^{13}\text{CO}_3$ (Mass Trace, Inc.) was used to prime the CO_2 /bicarbonate pool (Wolfe, 1992) and to determine basal CO_2 production. Immediately following the $\text{NaH}^{13}\text{CO}_3$ infusion, $[1-^{13}\text{C}]$ -palmitate solutions were infused at a constant rate (0.068 $\mu\text{mol } ^{13}\text{C}$ -palmitate $\cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) for 4 h. Breath and blood samples were collected during a basal, pre-infusion period, and when isotopic enrichment plateau was achieved (60–90 min for bicarbonate infusion and 120–180 min for palmitate infusion). One animal was infused with the palmitate-methyl- β -cyclodextrin mixture for 360 min with samples taken every 30 min for further verification that the plateau was achieved before the 120–180 min window. Additional samples collected to ensure that a plateau had been achieved (bicarbonate: t 30, 45, 60, 70, 80, 90 min; palmitate: t 30, 60, 90, 120, 135, 150, 165, 180 min) were not included in the calculations. Samples (breath and/or heparinized blood) were injected into evacuated vials for later measurement of $^{13}\text{CO}_2$ enrichments. Blood samples were collected into heparinized syringes and plasma was harvested after centrifugation for measurement of plasma enrichments of $[1-^{13}\text{C}]$ -palmitate. Enrichments of breath and plasma were determined by isotope ratio mass spectrometry (IRMS) and gas chromatography mass spectrometry (GCMS), respectively, (Metabolic Solutions, Inc., Merrimack, NH, USA).

2.6. Hepatocyte fatty acid oxidation

Isolation of porcine hepatocytes: Liver lobes were harvested from growing pigs (30–60 kg) to establish primary monolayer cultures of porcine liver cells (Caperna and Gavelek, 1994). Crossbred castrated male pigs (barrows) were maintained on a standard porcine diet and fed approximately 85% of ad libitum feed intake starting at 20 kg live mass. On the day prior to killing, pigs were given

unlimited access to feed. Pigs were stunned by a captive bolt device and exsanguinated. Livers were immediately excised, and the left lateral lobe was removed. Hepatocytes were isolated by the two-step collagenase perfusion technique essentially as previously described for the entire neonatal pig liver (Caperna et al., 1985) except only a small portion (approx. 80–100 g) of the lobe was used. A branch of the portal vein was cannulated and the cut surfaces of the lobe were sewn with 00 silk suture material to create back pressure during the perfusion. Collagenase buffer was modified to contain a 1:1 mixture of M199 and 100 mM HEPES-buffered saline containing 67 mM NaCl, 4.8 mM CaCl_2 and 6.7 mM KCl. The buffer mixture was amended with 0.1% BSA, 0.5% glucose, 0.075% collagenase and 5 $\mu\text{g}/\text{ml}$ bovine insulin.

Freshly isolated hepatocytes (4×10^6) were seeded into T-25 flasks (Costar, Corning, NY) coated with approximately 150 μg pig tail tendon collagen. Cells were initially maintained in a mixture of DMEM (low glucose)-M199 (1.25 g/l NaHCO_3) medium containing 10% fetal bovine serum (FBS), and amended with, β -mercaptoethanol (1×10^{-4} M), glutamine (2×10^{-3} M), penicillin (100 units/ml), streptomycin (0.1 mg/ml), gentamicin (50 $\mu\text{g}/\text{ml}$) and fungizone (amphotericin B, 1.25 $\mu\text{g}/\text{ml}$) at 37 °C and 5% CO_2 . Three hours after plating, monolayers were washed twice with HEPES-buffered saline and medium was replaced with supplemented DMEM-M199 medium containing 5% FBS. On the following day, monolayers were washed with HEPES-buffered saline and switched to serum-free M199 medium (M199 Hanks' salts: M199 Earle's salts and 1.25 g/l NaHCO_3 , 2:1 ratio) containing 25 mM HEPES, β -mercaptoethanol, glutamine, penicillin/streptomycin, fungizone, gentamicin and supplemented with 6.25 $\mu\text{g}/\text{ml}$ insulin, 6.25 $\mu\text{g}/\text{ml}$ transferrin, 6.25 ng/ml selenium, 1.25 mg/ml albumin and 5.35 $\mu\text{g}/\text{ml}$ linoleic acid (ITS+, Collaborative Biomedical Products, Bedford, MA, USA), 500 ng/ml porcine glucagon (G-3157, Sigma), 1% bovine serum lipids (C-5555, Sigma), 1% DMSO (D-2650, Sigma) and 1×10^{-6} M dexamethasone acetate. Cells were maintained in an air environment at 37 °C. Where noted, carnitine and betaine were added to the culture medium at the start of the serum-free culture period. Medium was changed daily. Except where noted, all

media components and reagents were purchased from GIBCO BRL (Gaithersburg, MD, USA).

In vitro palmitate oxidation: On the fourth day of culture, media was removed, cells were washed with HEPES-saline and fresh media (6 ml) without lipids but containing 2×10^{-5} M $1\text{-}^{13}\text{C}$ -palmitate, prepared in 20% MBCD (final concentration 0.2%), was added. Immediately after addition of medium, the standard cap on each flask was replaced with a cap containing a rubber septum (Wheaton, #240676, Millville, NJ) and each flask was tightly sealed and incubated at 37 °C. After 6 h, cells were killed and CO_2 was released into the flask headspace by addition of 2.5 ml of 20% TCA, via the septum using a syringe and 21 gauge needle. Following incubation at room temperature for 15 min, a 10 ml gas sample was removed and injected into an evacuated tube (Exetainer, Labco Ltd., Buckinghamshire, England) for the determination of $^{13}\text{CO}_2$ by mass spectrometry (IRMS, Metabolic Solutions, Nashua, NH). The ^{13}C atom percent enrichment (APE) of controls (cultures incubated with unlabeled palmitate) was subtracted from the APE of experimental cultures. The APE for controls in experiments reported was 1.0979 ± 0.0014 and 1.0981 ± 0.0008 for cultures without or with added carnitine, respectively. Preliminary in vitro studies conducted under similar conditions indicated that betaine did not influence total cell protein in hepatocyte cultures.

2.7. Mass spectrometry analysis

[$1\text{-}^{13}\text{C}$]-Palmitate enrichment in plasma, infusate and cell culture media was determined by gas chromatography-mass spectrometry (GCMS) on a Hewlett Packard 5989A system (Palo Alto, CA) using a Restek Rtx-1 capillary column. Extracted fatty acids were derivatized with addition of MTBSTFA: acetonitrile (1:1 v/v) to produce a t-BDMS derivative. Quantification of the natural and [$1\text{-}^{13}\text{C}$]-palmitate fatty acid derivatives was carried out by GCMS in the electron impact mode. $^{13}\text{CO}_2$ enrichment in breath and heparinized whole blood samples was determined by gas isotope ratio mass spectrometry (IRMS) on a Europa Scientific (Crewe, UK) 20/20 Stable Isotope Analyzer. The breath samples were loaded directly on the IRMS via a Europa Scientific ABCA sample purification system. Blood samples were first acidified with a saturated citric acid solution to liberate the carbon

dioxide and the released gas loaded on the IRMS in the same manner as the breath samples.

2.8. Calculations and statistics

The palmitate entry rate [$R_a(\text{palmitate})$], the CO_2 production rate [$R_a(\text{CO}_2)$] during the bicarbonate infusion, the palmitate oxidation rate and the percent of palmitate uptake oxidized were calculated as follows (Wolfe, 1992):

$$R_a(\text{palmitate}) = [(E_{\text{ip}}/E_{\text{palm}}) - 1] \times F_{\text{palm}}$$

$$R_a(\text{CO}_2) = [(E_{\text{ib}}/E_{\text{pCO}_2}E_{\text{bCO}_2}) - 1] \times F_{\text{bicarb}}$$

$$\text{Palmitate oxidation} = [E_{\text{pCO}_2} \times R_a(\text{CO}_2)] / E_{\text{palm}}$$

$$\begin{aligned} \text{Palmitate uptake oxidized (\%)} \\ = [[E_{\text{pCO}_2} \times R_a(\text{CO}_2)] / F_{\text{palm}}] \times 100 \end{aligned}$$

where E_{ip} was the isotope enrichment of the ^{13}C -palmitate infusate, E_{palm} was the plateau enrichment of plasma palmitate, F_{palm} was the infusion rate of the ^{13}C -palmitate infusate, E_{ib} was the isotope enrichment of the ^{13}C -bicarbonate infusate, E_{bCO_2} was the plateau enrichment of plasma or breath CO_2 during the ^{13}C -bicarbonate infusion, F_{bicarb} was the infusion rate of the ^{13}C -bicarbonate infusate, and E_{pCO_2} was the plateau enrichment of plasma or breath CO_2 during the ^{13}C -palmitate infusion. Average plateau values were used in comparisons.

The primary comparisons of interest between fed (both growth and kinetic data) pigs were: (i) control vs. betaine (0.125 and 0.5%); (ii) 0.125% vs. 0.5% betaine and for the fasted (kinetic data) pigs; (iii) control vs. 0.5% betaine. To compare data from fed pigs, ANOVA was used. Unpaired *t*-tests were used to compare the fasted pigs. To determine the effect of betaine on response of fatty acid oxidation to fasting, the average plateau values were compared using paired Student's *t*-tests for the control and betaine (0.5%) pigs studied under both fed and fasted conditions. The level of significance was set at $P < 0.05$. Data in tables are expressed as mean \pm standard error of the mean (S.E.M.). For hepatocytes studies control and betaine-treated cultures were compared by paired Student's *t*-test analysis.

Table 2

The effect of betaine on growth performance of pigs housed in metabolism crates

	Control		Betaine 0.125%		Betaine 0.5%	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
	(n = 19)		(n = 15)		(n = 15)	
Pre-treatment (control diet)						
ADG (kg/d)	0.57	0.05	0.55	0.07	0.55	0.05
ADFI (kg/d)	1.77	0.05	1.75	0.04	1.78	0.06
F:G	3.45	0.35	3.40	0.30	3.41	0.42
Treatment diet						
ADG ^a (kg/d)	0.67	0.05	0.81	0.03	0.83	0.08
ADFI (kg/d)	1.88	0.05	1.91	0.04	1.96	0.07
F:G ^a	3.41	0.52	2.39	0.10	2.55	0.26

Abbreviations: ADG = Average daily gain; ADFI = Average daily feed intake; F:G = Feed to gain ratio (kilogram of feed required per kg of body weight gain).

^a Betaine groups different from control ($P < 0.05$).

3. Results

3.1. Growth performance

The growth performance responses are presented in Table 2. While housed in metabolism crates, all pigs grew at similar rates during the pre-treatment period (consuming the control diet). Once switched to diets containing betaine (0.125 and 0.5%), pigs grew on average more than 20% faster than did the animals that remained on the control diet ($P < 0.05$), despite consuming a similar amount of feed to the control animals. The feed conversion efficiency (feed to gain ratio) of the pigs fed betaine improved 25% or more as compared to controls ($P < 0.05$).

3.2. Blood parameters

Plasma concentrations of non-esterified fatty acids (NEFA) and plasma urea nitrogen (PUN) are presented in Table 3. Dietary betaine had no

effect on NEFA levels during either a fed or fasting state. Likewise, PUN levels were similar among treatment groups.

3.3. In vivo whole-body fatty acid oxidation

Fatty acid oxidation data is presented in Table 4. Fasting increased palmitate oxidation rates for both treatment groups. On average, fasting increased the rate of palmitate oxidation eight-fold ($P < 0.05$) and the percent of palmitate uptake oxidized four-fold ($P < 0.01$). Dietary betaine did not affect this response of the pig to fasting. The CO_2 entry rate during the fed state tended to be lower in pigs consuming betaine ($P < 0.10$), but all other parameters were similar between treatments.

3.4. In vitro hepatic fatty acid oxidation

The presence of added carnitine had a marked influence on ^{13}C -palmitate oxidation in monolayers

Table 3

The effect of dietary betaine on plasma non-esterified fatty acids (NEFA) and urea nitrogen (PUN) levels in pigs

	Control			Betaine 0.125%			Betaine 0.5%		
	Mean	S.E.M.	n	Mean	S.E.M.	n	Mean	S.E.M.	n
NEFA ($\mu\text{Eq/l}$)									
Fed	125.6	19.9	13	94.3	9.0	13	100.3	6.5	13
Fasted	345.6	116.3	5	—	—	—	380.4	155.8	5
PUN (mg/ml)									
Fed	11.4	0.8	11	9.6	0.9	10	10.0	0.8	12
Fasted	9.2	0.4	5	—	—	—	9.3	1.3	5

Table 4

Effect of dietary betaine on whole body palmitate entry rate [R_a (palm)] and palmitate oxidation

	Control				Betaine (0.5%)			
	Fed		Fasted		Fed		Fasted	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
	(n=4)		(n=5)		(n=4)		(n=5)	
Wt., kg	50.5	0.9	53.4	3.0	53.6	2.1	56.1	4.5
R_a (palm), $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$	1.72	0.35	2.96	0.68	2.03	0.90	3.20	0.91
R_a (CO_2), $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1\text{b}}$	1008 ^a	87	626	17	809 ^a	47	594	76
Palmitate oxidation, $\mu\text{mol min}^{-1} \text{kg}^{-1\text{c}}$	0.170	0.020	1.235	0.348	0.128	0.025	1.074	0.462
% Palmitate uptake oxidized ^b	13.7	2.0	52.2	6.4	9.7	2.3	40.5	8.2

^a Betaine group tended to be different from control ($P<0.10$).^b Fasted group was different from fed group ($P<0.01$).^c Fasted group was different from fed group ($P<0.05$).

of porcine hepatocytes (Fig. 1). Maximal stimulation of oxidation was observed at approximately 0.5 mM carnitine. Addition of 2 mM betaine to monolayers of porcine hepatocytes for 3 days had no influence ($P>0.1$) on ^{13}C -palmitate oxidation in the absence or presence of 1 mM carnitine (Fig. 2).

4. Discussion

In the present study, dietary betaine enhanced the rate of body mass gain and the feed efficiency of female crossbred pigs in the 40–60 kg mass range, compared to similarly treated controls. These findings were unexpected as earlier studies, including those from our laboratory, have not

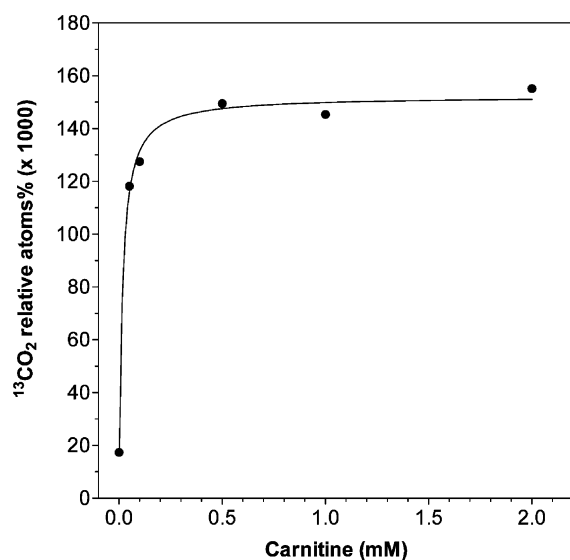


Fig. 1. The influence of carnitine on the oxidation of ^{13}C palmitate in porcine hepatocyte monolayers. Hepatocytes were prepared and maintained in culture as described in Section 2. Carnitine was added to flasks at various levels between 0.05 M and 2 mM for the duration of the culture period. Data represent the mean of duplicate flasks prepared from one pig.

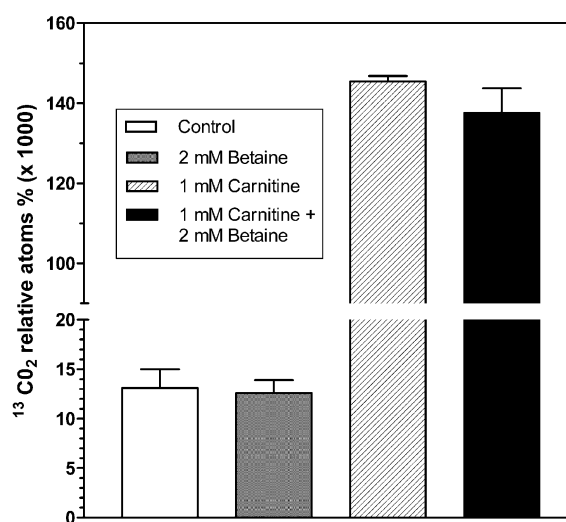


Fig. 2. The influence of carnitine and betaine on the oxidation of ^{13}C palmitate in porcine hepatocyte monolayers. Hepatocytes were prepared and maintained in culture as described in Section 2. Where noted, 1 mM carnitine and 2 mM betaine were added to the culture flasks for the duration of the culture period. Data represent the mean \pm S.E.M. of duplicate cultures from five different pigs.

previously demonstrated growth enhancement in animals fed a variety of experimental diets containing similar levels of betaine (Cadogan et al., 1993; Matthews et al., 1998; Overland et al., 1999; Matthews et al., 2001a,b; Fernández-Fígares et al., 2002; Lawrence et al., 2002).

A new pig growth trial from Finland has recently been published reporting improved feed conversion ratios and daily gains with lower level of dietary betaine (Siljander-Rasi et al., 2003). In that study, the feed intake was restricted by a similar degree to that in the current report (80–85% of ad libitum). In the Finnish study, feed efficiency and growth rates improved with increasing level of dietary betaine (for the two highest levels). The levels of betaine used in the Finnish study were lower than used in other published trials. It is possible that the most effective dose is lower than that used in the current study and in other studies. In the current study, the 0.5% betaine diet showed no further improvement in growth performance over the 0.125% betaine diet. Perhaps the 0.5% dose was beyond the break point of the response curve. However, this hypothesis is not supported by results from our earlier growth trial (Fernández-Fígares et al., 2002) where we observed a linear reduction in carcass fat (per kg of dry matter) from 0% to 0.5% dietary betaine.

Several potential factors may have contributed to the growth performance findings in the current study. Most growth trials take place over many weeks with ad libitum feeding and in conditions that are potentially less stressful than found in production. The time frame of this study was relatively short (under 2 weeks). The pigs were restrictively fed (80% ad libitum) and housed in a very confined space (metabolism crates). In trials in which pigs are given ad libitum access to feed, a tendency towards a reduction in voluntary feed intake has been reported, accompanied by no change in overall growth (Matthews et al., 1998, 2001a). In the current experiment, where feed intake was strictly controlled and limited to 80% of expected ad libitum intake, the improvement in feed:gain ratio resulted in the overall observed increase in growth rate.

Gender may have also played a role in the growth results of the current study. Only females were used in the current growth study, while we used castrated males in our previous growth trial (Fernández-Fígares et al., 2002). When male and female pigs have been compared directly, conflict-

ing results have been previously obtained with respect to ability of betaine to alter growth and lipid metabolism (Lawrence et al., 2002). The previously noted decrease in voluntary feed intake has been reported in betaine growth trials with females alone or with groups of mixed sexes (Matthews et al., 1998, 2001a). The Finnish study used mixed genders (females and castrated males), but they did not report their data by gender (Siljander-Rasi et al., 2003).

We cannot rule out that the observed growth effect associated with betaine addition in this study is relatively transient and may be masked or attenuated in longer growth trials. Other reported studies with nutritionally adequate diets and similar levels of betaine, have been long-term evaluations. Even when separated into more restrictive time periods, these have still been considerably longer than the time frame of the current study (Matthews et al., 1998; Lawrence et al., 2002). Finally, the animals in the current experiment were housed in metabolism cages. While the limited physical movement might be associated with reduced energy expenditure, the pigs might also be exposed to a higher level of stress due to artificial confinement and feed restriction, compared to animals maintained either in group pens or in larger pens on ad libitum feeding. It has been previously suggested that betaine has positive effects on growth and metabolism in animals that are exposed to various physiological stresses (Virtanen 1995; Barak et al., 1997; Kidd et al., 1997; Junnila et al., 2000).

The improvement in growth performance may have been accompanied by a better utilization of dietary protein, as suggested by the tendency for lower concentrations of PUN with addition of betaine to the diet ($P < 0.11$). Matthews et al. (1998) observed decreased serum urea N levels under some conditions with dietary betaine, but others have reported no effect of betaine on urea N levels in blood (Matthews et al., 2001a; Fernández-Fígares et al., 2002). Although we did not detect changes in PUN in our earlier growth trial, we did observe a tendency for increased efficiency for lean gain (Fernández-Fígares et al., 2002).

In the present study, we found betaine to have no significant effect on palmitate oxidation, either in vivo or in vitro. Although palmitate oxidation was significantly enhanced by fasting, dietary betaine (0.5%) did not influence palmitate oxidation or utilization in either the fasted or fed state. In

the hepatocyte model, it is clear that available carnitine markedly limits the rate of palmitate oxidation and this may indicate that at least following 4 days of culture, cellular levels of carnitine are attenuated. In other hepatocyte studies where fatty acid utilization has been investigated, carnitine has been routinely added to the extracellular environment presumably to ensure maximal rates of oxidation (Pegorier et al., 1983; Odle et al., 1991). When betaine was added to carnitine-free and carnitine amended cultures, no enhancement of oxidation was observed. In addition, since these cultures appear to be highly sensitive to levels of carnitine, the data would suggest that betaine had no influence on intracellular levels of carnitine. In the presence of high carnitine (1 mM), varying the levels of insulin and glucagon had only marginal effects on palmitate oxidation (data not shown; see also Pegorier et al., 1983) which suggests that at least in porcine hepatocyte cultures maintained in complete serum-free medium, carnitine levels, and, therefore, fatty acid entry into mitochondria are the key regulators of fatty acid oxidation.

Plasma levels of NEFA observed in this study were also unaffected by betaine. It is possible that lipid metabolism was not affected by betaine in these animals; however, given that growth and feed efficiency were affected, we predict that these pigs were accruing less body fat. If this was the case, it may be more likely that betaine's effect on lipid metabolism in the pig is not due to its potential effects on oxidation; rather, decreased adipose accretion may be a result of reduced lipogenesis. Another possibility is that different tissues and/or adipose depots may respond differently to betaine and these localized changes would likely be undetectable on a whole body basis.

In conclusion, betaine appears to enhance growth performance of pigs that are housed in a confined space and limit-fed. Our previously observed effects of betaine on lipid accretion (Fernández-Fígares et al., 2002) appear not to be due to an enhancement in fatty acid oxidation, measured either in vivo or in vitro. It is possible that lipid synthesis was affected by betaine, but this requires further study. Betaine may be useful as a partitioning agent, especially when limit feeding is employed and/or housing conditions are stressful. However, further work will be required to establish the relationship of protein and lipid turnover under these conditions.

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